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Relationship between Structure, Conformation, and Antischistosomal Activity of Nitroheterocyclic Compounds

C. H. ROBINSON, E. BUEDING, AND J. FISHER

Department of Pharmacology and Experimental Therapeutics, School of Medicine, and Department of Pathobiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

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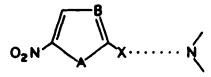
SUMMARY

The biochemical and chemotherapeutic effects of a number of nitroheterocyclic compounds, including niridazole [1-(5-nitro-2-thiazolyl)-2-imidazolidinone] and some 5-nitro-2-furyl derivatives, on *Schistosoma mansoni* were investigated. Those compounds which had marked schistosomicidal activity had common structural and conformational features. These comprised a 5-nitrothiazolyl or 5-nitrofuryl ring, with a nitrogen substituent (generally part of an amide or oxadiazole system) attached to C-2 via a rigid side chain. The latter contains either a C=C double bond or a nitrogen atom attached directly to C-2 of the heterocyclic ring. These features, which are illustrated in the accompanying structure, are believed to be



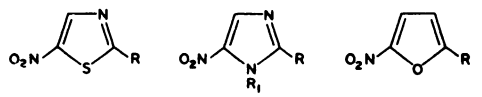
A=O and B=CH or A=S and B=N

X=N (or CH=CH)

essential for significant antischistosomal activity. The schistosomicidal effects of compounds of this structural type were invariably preceded by a reduction of phosphorylase phosphatase activity in the worms, suggesting a common mode of action.

INTRODUCTION

Many nitro-substituted heterocyclic compounds, e.g., nitrothiazoles (a), nitroimidazoles (b), and nitrofurans (c), have antibacterial and antiprotozoal properties, but only very few of them are endowed with antischistosomal activity (2, 3). Among the latter, the most prominent one is the nitrothiazole derivative 1-(5-nitro-2-thiazolyl)- 2- imidazolidinone (niridazole) (I), whose antischistosomal activity in animals and in man has been well documented (4–6). However, the vast majority of antiprotozoal nitroheterocyclic compounds are



a b c

completely devoid of antischistosomal activity. Therefore, the structural features conferring antischistosomal activity appear to be far more selective and specific than

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the structural requirements for antiprotozoal activity of heterocyclic compounds.

If the antischistosomal activity of niridazole were determined by specific structural characteristics, compounds endowed with similar properties not only should have antischistosomal action but also should interfere with the same biochemical and physiological mechanisms in the parasite. Studies reported in this paper indeed have revealed a close relationship between structure, conformation, and the biochemical antischistosomal effects of nitro- and nitroso-substituted heterocyclic and aromatic compounds.

EXPERIMENTAL PROCEDURE

Adult schistosomes were obtained in the same manner as in a previous study (7). Oral administration of the compounds tested was initiated 7–8½ weeks after the mice had been infected with cercariae of *Schistosoma mansoni*. The compounds were either administered in a diet mixture or fed by stomach tube in a suspension of gum acacia (0.5%, w/v).

Glycogen determinations. Glycogen levels of the worms were determined by either of two enzymatic methods. One, involving the use of glycogen phosphorylase and α-1,6-amyloglucosidase, had been found to be highly specific (8, 9), but the need for preparing these enzymes at relatively frequent intervals (crystalline rabbit muscle phosphorylase *b* may lose its activity at any time within 30–60 days after its preparation) led us to test the usefulness of a commercially available product, Diazyme (Miles Laboratories, Marshall Division, Elkhart, Ind.), containing a 1,4-, 1,6-α-amyloglucosidase, which catalyzes the degradation of glycogen to glucose. This preparation, combined with glucose oxidase and horseradish peroxidase, has been used by Johnson and Fusario (10) for the determination of glycogen in mammalian tissues. A modification of this method (10) and its adaptation for the spectrophotometric determination of glycogen in schistosomes, by coupling the catalytic activity of Diazyme with those of two other commercially available enzymes, hexokinase and glucose 6-phosphate dehydrogenase (Boehringer), and with NADP is described as follows. The dialyzed Diazyme solution, prepared according to Johnson and Fusario (10), was diluted 10-fold with 0.1 M potassium phosphate buffer, pH 6, before use. Following digestion of two male schistosomes in 0.1 ml of 1.0 N KOH and neutralization of the digest with 2.5 N H_3PO_4 to pH 7.0–7.5 (8), the mixture was brought to a volume of 0.3 ml with water and was centrifuged for 10 min at 2000 × *g*. Degradation of glycogen and subsequent spectrophotometric determination of glucose were then allowed to proceed in the same cuvette. To 50 μl of the supernatant fraction of the neutralized digest were added, with mixing, 0.1 ml of the dilute dialyzed Diazyme solution and 10 μl of 1.0 N HCl. After incubation of the mixture at 37° for 90 min, the following two solutions were added: (a) 0.3 ml containing 0.3 μmole of NADP, 3.75 μmoles of ATP, and 3 μmoles of $MgCl_2$, and (b) 0.3 ml of 0.27 M glycylglycine buffer, pH 8.3. After mixing and recording the optical density at a wavelength of 340 mμ, 0.04 ml of 0.05 M glycylglycine buffer (pH 7.4) containing 0.5 unit of hexokinase and 0.28 unit of glucose 6-phosphate dehydrogenase was added with mixing. After 15 min the optical density was recorded again. The glycogen content of the sample was calculated on the basis of the molar extinction of NADPH ($\epsilon = 6220\ M^{-1}\ cm^{-1}$). Determination of glycogen in 26 digests of worms revealed a close agreement between the two methods, with variations never exceeding ±4%.

Glycogen phosphorylase measurements. Glycogen phosphorylase activity and phosphorylase inactivation in schistosome homogenates, catalyzed by a phosphorylase phosphatase, were assayed as described previously (7). Glycogen phosphorylase activity was determined (a) at zero time, i.e., immediately following homogenization of the worms in 20% glycerol–0.05 M glycylglycine buffer (pH 7.4), (b) 15 min, and (c) 30 min after incubation of the homogenate at 20°. The initial glycogen phosphorylase activity of schistosome homogenates amounted to 7–9.5 mμmoles of

glucose 1-phosphate produced per minute per milligram of protein. In worm homogenates from control animals, phosphorylase activity was reduced approximately one-half after 15 min. After 30 min, residual phosphorylase activity amounted to only 20–25% of the activity present before incubation. In schistosomes obtained from mice which had received the compounds tested in this study, phosphorylase inactivation was determined simultaneously with that of worms from control animals which had been infected on the same day by the same number of cercariae, but to which no compound had been administered. The percentage inhibition of phosphorylase phosphatase activity in the experimental group was calculated by the use of the formula $[(D_C - D_E)/D_C] \times 100$, where D_C is the observed percentage decrease in glycogen phosphorylase activity after incubation of the homogenate of control worms for 15 min at 30° and D_E is the corresponding percentage decrease in phosphorylase activity of the homogenate of worms from the experimental group. The percentage increase in residual phosphorylase activity in the experimental group was calculated by using the formula $[(R_E - R_C)/R_C] \times 100$, where R_E and R_C are the observed percentage residual phosphorylase activities after incubation for 30 min (30°) of the homogenates of the experimental and control groups, respectively.

The percentage of damage to the female reproductive system of schistosomes, demonstrable by means of an *intra vitam* stain, was evaluated according to a recently reported scoring procedure.[1]

Synthesis of m-nitrocinnamic acid N-isopropylamide (XVI). *m*-Nitrocinnamic acid (2.0 g) was dissolved, with vigorous stirring, in oxalyl chloride (40 ml). Evaporation of this solution under vacuum, at room temperature, gave the crude acid chloride, which was dissolved in benzene (20 ml). This benzene solution was cooled in an ice bath, and isopropylamine (10 ml) was added dropwise, with stirring. Water was added, and the benzene layer was washed first with 2 N hydrochloric acid and then with water, and was dried (anhydrous Na_2SO_4) and evaporated under vacuum. The crude, solid product was crystallized from aqueous methanol to give *m*-nitrocinnamic acid *N*-isopropylamide as colorless needles (1.79 g), m.p. 118–120°; infrared spectrum, λ_{CHCl_3} 2.89, 2.99 (NH), 5.98 μ (amide CO); NMR spectrum (deuterochloroform, with tetramethylsilane as internal reference), 1.26δ (6H, doublet, J = 7 Hz, $CH(CH_3)_2$), 4.18δ (1H, septet, $CH(CH_3)_2$), 6.28δ (1H, broad singlet, NH), 6.65δ (1H, doublet, J = 16 Hz), and 7.63δ (1H, doublet, J = 16 Hz) due to *trans*-CH=CH, 7.25–8.32δ (4H, multiplet, aromatic H); mass spectrum, *m/e* 234 (M^+), 191 ($M-CH(CH_3)_2$), 176 ($M-NHCH(CH_3)_2$).

	$C_{12}H_{14}N_2O_3$
Calculated:	C 61.53, H 5.98, N 11.97
Found:	C 61.73, H 6.02, N 12.07

The infrared spectrum was measured in chloroform solution on a Perkin-Elmer Infracord instrument, and the NMR spectrum was obtained with a Varian A-60 spectrometer. NMR chemical shifts are on the δ-scale (tetramethylsilane = 0).

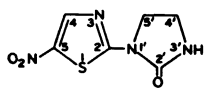
RESULTS

One of the first effects of antischistosomal compounds is the hepatic shift, in which the worms lose their attachment to the internal wall of the mesenteric veins and are carried to the liver by the venous blood flow (11, 12). In the case of niridazole the worms begin to shift toward the liver 72 hr following the administration of this drug to the host (7). However, earlier changes in the parasites are observable with this compound. Even 18–24 hr after its administration, the phosphorylase phosphatase activity of the worms is reduced; this is followed by a decrease in the glycogen levels and an activation of glycogen phosphorylase, and is associated with the appearance of abnormalities in the female reproductive system (7). The latter effect is also observed with many other antischistosomal compounds, but the inhibition of phosphorylase phosphatase activity pre-

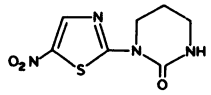
[1] J. G. Bourgeois and E. Bueding, unpublished observations.

ceding the hepatic shift is characteristic for niridazole, because no significant change in phosphorylase phosphatase activity was observed even after a hepatic shift had been produced by the administration of trivalent organic antimonials.

Structural features necessary for antischistosomal activity in niridazole (I)

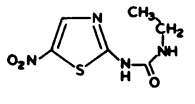


I

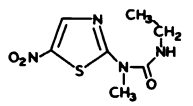


II

include the nitro group and the nitrogen (N-3′) of the imidazolidinone ring (13). It has been reported that alkylation of the latter nitrogen results in partial loss of activity (14). Enlargement of the imidazolidinone moiety to a 6-membered ring (II) had no adverse effect on activity (13, 14). Interestingly, the acyclic analogues III and IV have been found to be inactive



III



IV

(14). This suggests that the rigidified compound I may be locked into some biologically preferred conformation not readily accessible in the open-chain compounds III and IV. Examination of space-filling models of I revealed considerable steric crowding around the C—N bond linking the thiazole and imidazolidinone rings. While it seems probable that the rings are not coplanar, we believe that the precise deviation from coplanarity cannot be estimated accurately from models. Clearly, the actual angle between the planes of the rings varies with the degree of overlap of the p-electrons on N-1′ in niridazole both with the π-system in the thiazole ring and with the ureide grouping. The extent of these electronic interactions may well be important in determining antischistosomal activity.

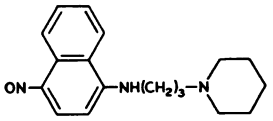
It has been reported by Werbel *et al.* (15) that a number of *N*-(dialkylamino-alkyl)-1-nitroso-4-naphthylamines have antischistosomal activity. 1-{3-[(4-Nitroso-1-naphthyl)amino]propyl}piperidine (V) was

TABLE 1

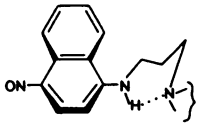
Effect of administration of a nitrosonaphthylamine (V) on S. mansoni

Mice infected with *S. mansoni* were fed a diet containing 0.25% of compound V. Worms from control mice fed the same powdered diet, but without the nitrosonaphthylamine, were used as controls. In the experimental group a hepatic shift began 10 days after the drug diet had been initiated.

Days after start of drug diet	Inhibition of phosphorylase phosphatase activity	Increase in residual phosphorylase activity (30 min: 30°)	Decrease in glycogen level	Damage to female reproductive system
	%	%	%	%
2	0	0	0	0
3	20	32	8	8
4	31	49	14	13
6	36	57	31	44
9	49	76	46	39
10	62	98	52	56



V



VI

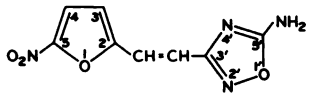
found by these authors to be among the most active ones within this group of compounds. When the latter was administered with the diet in a concentration of 0.25% to mice infected with *S. mansoni*, the time course of the biochemical and morphological changes in the worms was the same as those with niridazole. Prior to the hepatic shift there was an inhibition of phosphorylase phosphatase activity associated with changes in the female reproductive system of the worms, detectable by an *intra vitam* staining technique. This was followed shortly by a decrease in the glycogen levels of the male worms (Table 1). The hepatic shift of the schistosomes did not occur until 7 days after the onset of these changes. The identity of this sequence

with that observed following the administration of niridazole suggested that the mode of the antischistosomal action of the nitrosonaphthylamine (V) might be identical with that of niridazole.

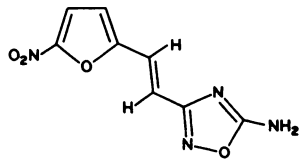
Examination of Courtauld space-filling models of the nitroso compound V and of niridazole (I) revealed interesting conformational similarities (Fig. 1a). The nitroso group and the naphthylamine nitrogen in V are essentially superimposable with the nitro group and N-1′, respectively, of niridazole. Furthermore, if the piperidine nitrogen in V were intramolecularly hydrogen-bonded to the naphthylamino group (see perspective drawing VI) in a quasi-6-membered ring, the piperidine nitrogen of V would be approximately superimposable on N-3′ of niridazole (I).

In contrast to many nitroheterocyclic compounds, a nitrofuran derivative, *trans*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole (VII) (SQ 18,506), synthesized recently by Breuer (16), shares similar conformational characteristics with niridazole (I).

We assume that the conjugated system of VII is in the planar, fully *transoid* form



VII



VIII

(see VIII), with maximum overlap of the π-electrons of the unsaturated side chain, maximum charge separation, and minimal nonbonded interactions. Comparison of space-filling models of niridazole and of compound VII in the planar, fully *transoid* conformation VIII shows that, if the respective nitro groups and aromatic rings are superimposed, the ethylenic CH=CH of compound VII is superimposable on the 1′-nitrogen of niridazole; i.e., the 1′-nitrogen atom falls at approximately the center of the C=C vinyl bridge (Fig. 1b). It appears that one of the key structural components in this type of antischistosomal compound is a nitrogen, with its unshared *p*-electrons, or a C=C double bond, with the associated π-electrons. In addition, given the superimposition described above, the 4′-nitrogen of the nitrofuryl compound (VII) is approximately coincident with the 3′-nitrogen of niridazole. In an attempt to determine whether there is a relationship between these conformational characteristics and antischistosomal activity, the effect of SQ 18,506 (VII) administered to mice infected with *S. mansoni* was tested. The time course of the effects of this compound on the worms was similar to that of niridazole and of the nitrosonaphthylamine (V). Shortly after the administration of the drug diet was initiated, there was a reduction of glycogen phosphorylase inactivation in the male worms; this was associated with a decrease in the glycogen levels and damage to the female reproductive system (Table 2). The decrease in phosphorylase inactivation was reflected in an increase in phosphorylase activity remaining after incubation of schistosome homogenates for 30 min at 30° (Table 2). Under these conditions, only approximately 25% of the original activity was detectable in homogenates of worms removed from untreated mice. As the drug diet was continued, these changes became more pronounced and were followed by a hepatic shift, which usually was observed 2–3 days after the initiation

FIG. 1. *Courtauld models of niridazole, SQ 18,506 and related compounds*

a. Top, niridazole (I); bottom, nitrosonaphthylamine (V). b. Top, niridazole (I); bottom, SQ 18,506 (VII). c. Top, SQ 18,506 (VII); bottom, isomer of SQ 18,506 (IX). d. Top, nitrofurylacrylamide (XIa); bottom, SQ 18,506 (VII).

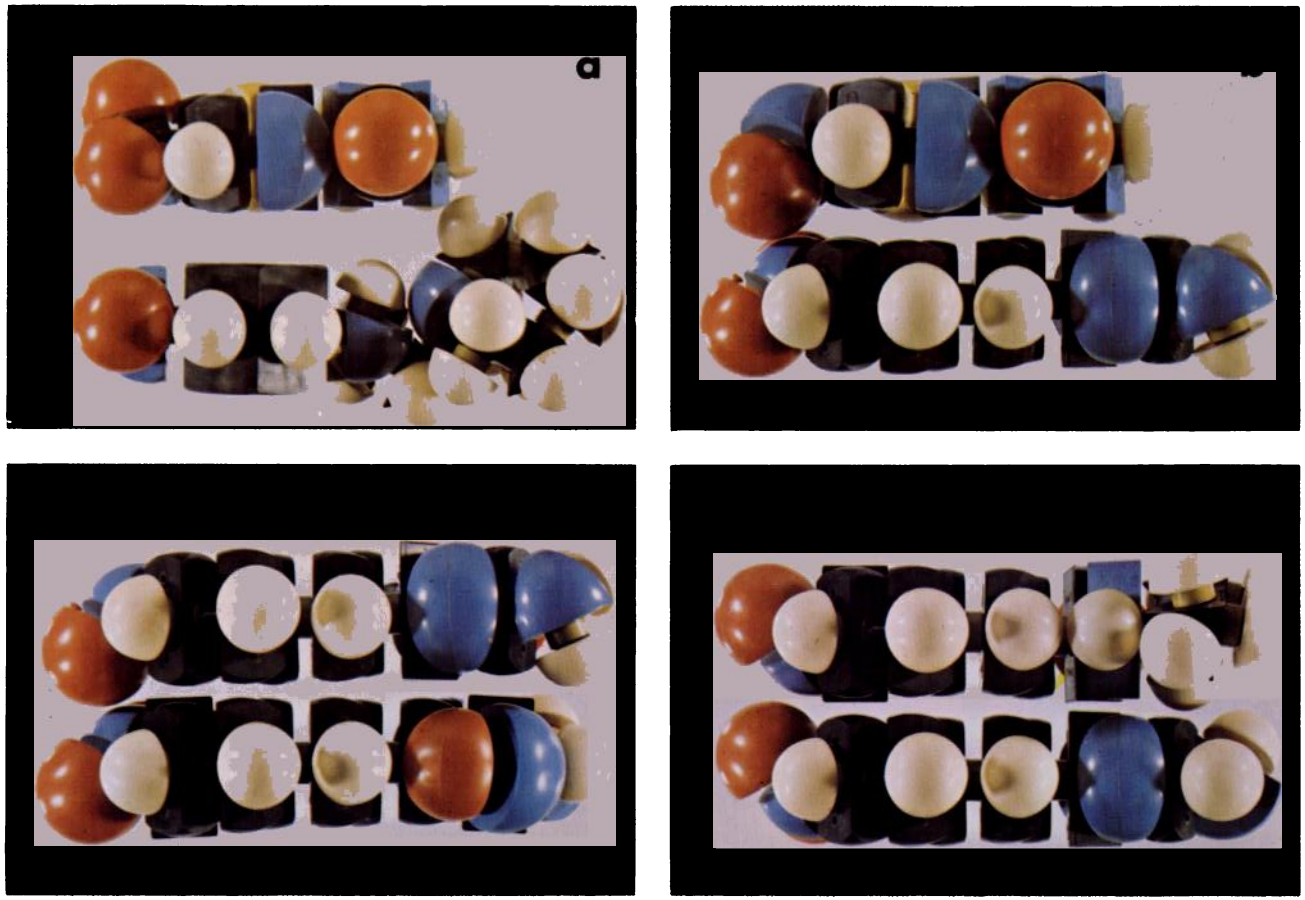


FIG. 1

TABLE 2

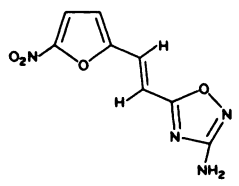
Effect of SQ 18,506 (VII) on S. mansoni

The compound was administered to mice infected with *S. mansoni*.

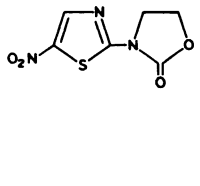
SQ 18,506 in diet	Duration of drug diet administration	Drug diet discontinued	Inhibition of phosphorylase phosphatase activity	Increase in residual phosphorylase activity (30 min; 30°)	Increase in phosphorylase activity	Decrease in glycogen level	Damage to female reproductive system
%	*days*	*days*	%	%	%	%	%
0.3	3	0	54	202	26	49	35
		1	60	232	31	62	51
		2	66	250	38	53	48
		3	50	70	29	32	42
1.2	1	0	27	84	0	25	25
		1	22	84	12	49	30
		2	30	210	17	38	36
		4	15	78	22	28	36
		8	18	62	28	14	50
1.2	2	0	45	168	15	58	100
		1	70	256	21	78	100
		2	79	307	27	78	85
		4	56	200	31	58	100
		8	82	510	26	75	100
1.2	3	0	68	332	42	70	100
		1	78	350	30	83	100
		3	56	272	42	61	100
		4	75	305	62	93	100

of the diet containing 1.2% of the compound. Compared with the nitrosonaphthylamine V, the onset and intensity of these changes were much more marked with the nitrofuran. This was also reflected in the higher antischistosomal activity of the latter compound. A relationship between the dose and the effect of this compound on the worms was indicated by the observation that the greater the dose or the duration of drug administration, the greater were these effects on the worms. The time course of these changes was similar to that observed with niridazole (I) (7). As with the latter, the effects on the worms generally reached their maxima 1–3 days after the administration of a subcurative dose had been discontinued.

The chemotherapeutic activity of SQ 18,506 (VII) was found to be considerable. Administration of a diet containing 1.2% of the compound for 5 days, or 0.3% for 8 days, to mice infected with *S. mansoni* consistently resulted in the complete elimination of the worms (1). These and other antischistosomal effects of SQ 18,506 will be reported in detail elsewhere.



IX



X

The availability of an isomer of SQ 18,506 provided an opportunity to explore the extent of structural specificity in this series. In this isomer, IX (SQ 18,116), assuming a coplanar, fully *transoid* conjugated system, an oxygen atom now occupies the position of the 4′-nitrogen in

TABLE 3

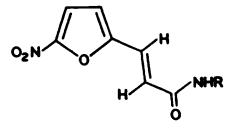
Effect on S. mansoni of SQ 18,116 (IX), an isomer of SQ 18,506, administered in diet to host

A diet containing SQ 18,116 in a concentration of 1.2% was fed for 5 days to mice infected with *S. mansoni*.

Days after discontinuation of drug diet	Inhibition of phosphorylase phosphatase activity	Increase in residual phosphorylase activity (30 min; 30°)	Decrease in glycogen level	Damage to female reproductive system
	%	%	%	%
1	23	48	15	58
3	26	53	24	71
8	8	18	12	25
13	3	0	0	15
21	0	0	0	7

SQ 18,506 (VII) (Fig. 1c). If the assumption is correct that IX has a preferred conformation, i.e., the coplanar, fully *transoid* form, free rotation of the oxadiazole ring is precluded. Compared with SQ 18,506 (VII), the isomer (IX) had extremely low antischistosomal activity. While a diet containing 1.2% SQ 18,506 fed for 5 days resulted in complete elimination of the worms, administration of the isomer, IX, in the diet in the same concentration and for the same period of time did not even significantly reduce the number of worms. There was a slight temporary hepatic shift (with 50% of the worms remaining in the mesenteric veins), preceded by a reduction in the glycogen levels, inhibition of phosphorylase phosphatase, and some abnormalities in the female reproductive system, but all these changes were reversed 21 days after the administration of the drug diet had been discontinued (Table 3).

Similar marked differences in activity were observed when the effects of administration of the two isomers in a small particle size (5 μ in diameter) by gavage were compared. With a dosage schedule which consistently produced parasitological cures with SQ 18,506 (VII), i.e., 0.5 g/kg twice daily for 5 days, there was no reduction in the number of worms when SQ 18,116 (IX) was used instead. Again, only a slight, transitory decrease in phosphorylase phosphatase activity and a small decrease in the glycogen levels of the parasite over a period of a few days were observed under these conditions. Therefore, the antischistosomal activity of this isomer is minimal when compared with that of SQ 18,506. We infer that the 4′-nitrogen of SQ 18,506 is required for full antischistosomal activity, and cannot be replaced by



XI

a) R = $CH(CH_3)_2$

b) R = $CH_2COOC_2H_5$

TABLE 4

Effects of two nitrofurans (XIa and XIb) on S. mansoni

N-(5-Nitro-2-furanacryloyl)glycine ethyl ester (XIb) or *N*-isopropyl-5-nitrofuranacrylamide (XIa) was administered orally on 3 successive days at a daily dose of 250 mg/kg to mice infected with *S. mansoni*.

Days after discontinuation of drug	Inhibition of phosphorylase phosphatase activity		Increase in residual phosphorylase activity (30 min; 30°)		Decrease in glycogen level		Damage to female reproductive system	
	XIb	XIa	XIb	XIa	XIb	XIa	XIb	XIa
	%	%	%	%	%	%	%	%
1	28	42	60	88	32	49	31	42
3	34	53	71	112	48	64	52	57
5	22	39	46	78	31	53	45	55
8	7	23	18	44	10	24	38	49

oxygen. This inference is of interest in view of the report that replacement of the 3′-nitrogen of niridazole by oxygen (as in X) or by the —CH_2— grouping resulted in a loss of antischistosomal activity (14).

The association of antischistosomal activity with specific structural and conformational characteristics of the kinds discussed above is supported by consideration of the nitrofuran derivatives XIa and XIb. These compounds have been reported to be effective in the treatment of schistosomiasis by Lei *et al.* (17).

A mode of action common to both compounds XIa and XIb and niridazole (I) is indicated by the similarity of their effects on the worms and their time course when they were administered to mice infected with *S. mansoni* (Table 4). However, with the isopropyl derivative XIa, parasitological cures were obtained quite consistently only with a dosage schedule close to the LD_{50}. Furthermore, with the same dosage schedule, administration of the glycyl ester derivative XIb resulted in a reduction in the number of worms, but only occasionally in their complete elimination (Table 5). The higher potency of the isopropyl derivative XIa is also reflected in its more pronounced effects on the worms following administration of subcurative doses of the two compounds (Table 4).

Space-filling models, constructed on the basis of a planar, fully *transoid* system, show that the amide nitrogen in compounds XIa and XIb is coincident in space with the 4′-nitrogen of SQ 18,506 if the nitro groups and side chain CH=CH groups are superimposed (Fig. 1d). Similarly, the amide nitrogens in compounds XIa and XIb also correspond well to the 3′-nitrogen of niridazole.

Two nitrothiazole derivatives (XII and XIII) recently synthesized by Asato *et al.* (18) are also of interest. Although these compounds appear to have the key structural and conformational characteristics of niridazole and SQ 18,506, their administration did not result in a reduction of phosphorylase phosphatase activity or glycogen stores, or in a hepatic shift of the worms. It is possible that the basicity of the terminal nitrogen substituent is important in determining antischistosomal activity.

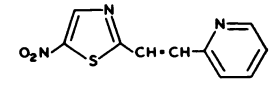
TABLE 5

Chemotherapeutic effects of two nitrofurans (XIa and XIb) on experimental schistosomiasis

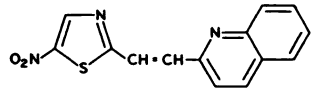
N-Isopropyl-5-nitrofuranacrylamide (XIa) or *N*-(5-nitro-2-furanacryloyl)glycine ethyl ester (XIb) was administered orally once daily to mice infected with *S. mansoni* at the following schedule: 250 mg/kg for 6 days, followed by 1 g/kg for 3 days, followed by 500 mg/kg for 5 days. Autopsies were performed 31–47 days after the last dose.

Nitrofuran	Total No. of mice	No. of surviving mice	Average reduction in No. of worms	Parasitological cures[a]
			%	%
XIa	30	14	98	86
XIb	30	26	61	12

[a] A mouse was considered parasitologically cured when not a single live worm could be recovered.

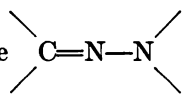


XII



XIII

While compounds XII and XIII lacked the type of antischistosomal activity characteristic for niridazole and SQ 18,506, their administration resulted in marked abnormalities in the reproductive system of the female worms. These effects were reversed over a relatively long period of time (approximately 10 weeks) (Fig. 2). Similar effects, limited to the female reproductive system of the parasite, were observed with two other nitroheterocyclic compounds, nitrofurantoin (XIV), whose conformation differs from that of the niridazole group



because of the side chain

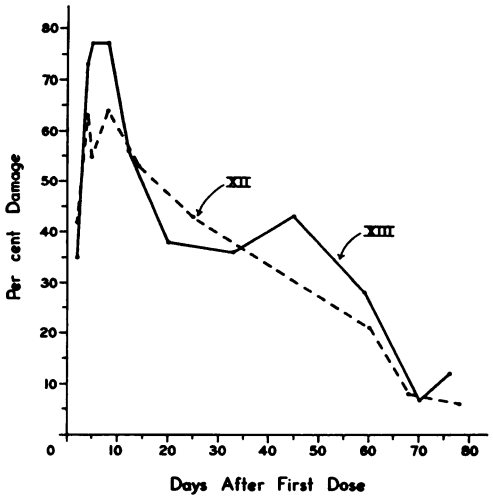
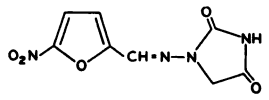
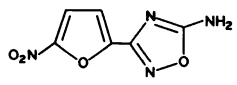


FIG. 2. *Effect of two nitrothiazoles on female reproductive system of S. mansoni*

The compounds were administered orally to mice infected with *S. mansoni*, the pyridine derivative (XII) at a daily dose of 0.5 g/kg for 6 successive days, the quinoline derivative (XIII) at a daily dose of 1 g/kg for 5 successive days. The abscissa shows days after the first dose; the ordinate, percentage of abnormalities in the female reproductive system.



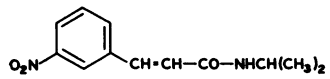
XIV



XV

with its different bond lengths and bond angles, and an analogue (SQ 18,646) (XV) of SQ 18,506 (VII), which lacks the vinyl bridge.

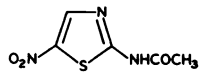
Therefore, it appears that a distinction must be made between two types of antischistosomal effects of nitroheterocyclic compounds. One is observed with many structurally unrelated compounds and is limited to reversible damage to the female reproductive system. The other type of antischistosomal action is initiated by specific biochemical effects, i.e., a reduction in phosphorylase phosphatase activity, followed by a hepatic shift and the eventual partial or complete elimination of the parasite. This second type of effect is exerted by compounds which have specific structural and conformational characteristics in common. In an attempt to define further these structural relationships with antischistosomal activity, an analogue (XVI) of the



XVI

active nitrofurylacrylic acid isopropylamide XIa was synthesized, in which the nitrofuran ring was replaced by the *m*-nitrophenyl group. This compound was completely devoid of antischistosomal activity at the highest tolerated dosage level. Therefore a nitrophenyl ring cannot replace a nitroheterocyclic ring for this type of antischistosomal activity.

According to Cuckler *et al.* (19), 2-acetamido-5-nitrothiazole (aminitrozole) (XVII)



XVII

has a slight degree of antischistosomal activity. We have confirmed the findings that prolonged administration of this compound at the highest tolerated dosage level results in a slight reduction in the number of worms. A hepatic shift occurred 2 weeks after a diet containing 0.2% of this compound was initiated, and this shift was preceded by a reduction in phosphorylase phosphatase activity and a moderate decrease of the glycogen stores of the worms (Table 6). Thus, qualitatively these effects and their time course were similar to those of niridazole and of SQ 18,506. Quantitatively, however, these antischistosomal effects were far less pronounced. This is also indicated by the report that administration of this compound results only in a reduction in the number of the parasites, but not in their complete elimination (19). As noted earlier, the 3′-nitrogen of niridazole (I) is required for full activity, and com-

TABLE 6

Effects of aminitrozole (XVII) on S. mansoni

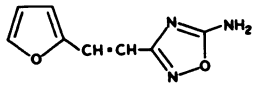
A diet containing 0.2% of this compound was administered for 15 days to mice infected with *S. mansoni*.

Days after initiation of drug diet	Inhibition of phosphorylase phosphatase activity	Increase in residual phosphorylase activity (30 min; 30°)	Decrease in glycogen level	Damage to female reproductive system
	%	%	%	%
1	0	0	0	7
2	0	0	0	4
3	8	11	6	18
4	15	28	11	26
6	22	37	19	34
11	38	132	31	55
13	35	118	28	63
15	48	192	37	72

pound XVII (which can be viewed as a niridazole analogue) lacks this key substituent, as well as the rigidifying effect of a ring.

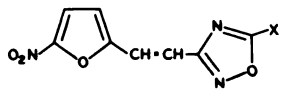
DISCUSSION

The studies with nitroheterocyclic compounds reported in this paper have indicated that antischistosomal activity is related to certain structural and conformational features. We wish to draw attention to additional criteria. For example, the nitro group of SQ 18,506 (VII) is essential



XVIII

for antischistosomal activity, as the desnitro compound XVIII was found to be inactive.[2] Similarly, desnitro niridazole lacks antischistosomal activity (14). On the other hand, modification of the amino substituent attached to the 5′-position of the oxadiazole ring of SQ 18,506 has no great effect on antischistosomal activity. Thus, each of the compounds XIXa–c, wherein the C-5′ substituent is methyl-



XIX

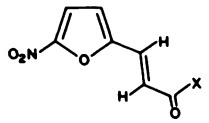
a) X = $NHCH_3$

b) X = $N(CH_3)_2$

c) X = $NHCOCH_3$

d) X = H

amino, dimethylamino, or acetamido, respectively, showed substantial activity.[2] It is particularly noteworthy that the desamino compound XIXd also had marked antischistosomal activity.[2] Furthermore, the amide nitrogen atoms in compounds XIa and XIb are essential for antischistosomal activity because the corresponding esters and thioesters XXa and b, in which nitrogen is replaced by oxygen and sulfur, respectively, have been reported to be inactive (19).



XX

a) X = OR

b) X = SR

c) X = NHNR

d) X = NHAr

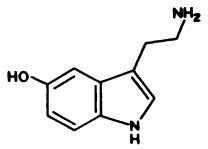
e) X = NR_2

It is also of interest that those compounds which have the amide nitrogen as part of a hydrazine or substituted hydrazine grouping (XXc), or which have an aryl substituent attached to nitrogen (XXd), have been found to be inactive (20). These two groups of compounds might be expected to have amide nitrogens slightly less basic than those of type XI. As pointed out already in connection with the nitrothiazoles XII and XIII, nitrogen basicity may be a critical factor in determining antischistosomal activity. However, compounds in which the amide nitrogen is tertiary (XXe) are also generally inactive (20), and these amide nitrogens might be expected to be somewhat more basic than those of secondary amides. In view of the intrinsically

[2] E. Bueding, I. Weliky, C. Náquira and G. Rose, unpublished observations.

very weak basicity of amide nitrogen, these effects might not be very substantial overall.

The antischistosomal activity of the compounds discussed in this paper may be related to their structural similarity to 5-hydroxytryptamine, whose concentration in *S. mansoni* is very high (21). Molecular models of 5-hydroxytryptamine (XXI) and the nitroheterocyclic antischistosomal agents suggest that, if the nitroheterocyclic compound (e.g., XIa) and 5-hydroxytryptamine (XXI) are juxtaposed so that the nitro group and phenolic hydroxyl group are superimposed, as well as the heterocyclic ring of XIa and the phenyl moiety of 5-hydroxytryptamine, then the ethylenic C=C double bond of XIa and the 2,3-double bond of the 5-membered ring of 5-hydroxytryptamine are also superimposed. The amide nitrogen of XIa and



XXI

the flexible side chain nitrogen of 5-hydroxytryptamine are also essentially superimposable, assuming a staggered side chain conformation for 5-hydroxytryptamine. It is pertinent that Dombro and Woolley (22) drew attention to the structural analogy between 5-hydroxytryptamine and cinnamic acid amides, and indeed showed that some cinnamamides were 5-hydroxytryptamine antagonists.

A connection between antischistosomal activity and 5-hydroxytryptamine would be of great interest, and work is in progress to test this hypothesis.

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